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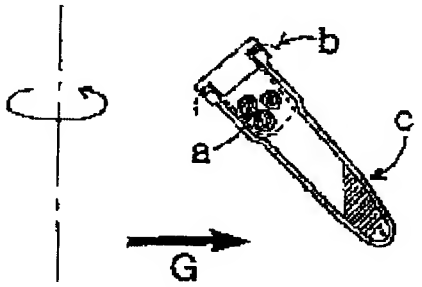
(54) METHOD FOR DETERMINING QUINOLENE AGENT

(57)Abstract:
PURPOSE: To obtain result of monitoring in a short time without requiring a highly advanced equipment by measuring luminescence which is generated by casting an excitation light to specimen containing quinolene agent under acid conditions.
CONSTITUTION: Keep 2-3 spherical absorbent waddings with a diameter which is approximately 5mm in a mouth, place the absorbent waddings (a) which fully contain saliva on a gauze (b) of a transparent sample tube (c), and then hold the gauze b, cover it with a lid, and then centrifuge it approximately for one minute, thus enabling a oral quinolene agent offxacine(OFLX) to be collected quantitatively. The OFLX is dissolved into 0.04M phosphor acid buffer liquid (pH3) with a concentration of 10μg/ml and then a saliva sample with pH3 whose fluorescent concentration is maximized is prepared. When a fluorescent lamp is cast to the saliva sample, color tone changes from a deep blue color due to reflection of illumination light to a yellow green color which can be distinguished vividly, thus enabling detection sensitivity of the quinolene agent to be enhanced when observing with naked eyes.



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CLAIMS

[Claim(s)]
[Claim 1] Assay of the quinolone agent characterized by measuring the fluorescence which irradiates excitation light and produces the analyte containing a quinolone agent as a result under acid conditions.
[Claim 2] Assay of the quinolone agent according to claim 1 characterized by the maximum fluorescence wavelength being near 490nm to 550nm at the time of acidity.
[Claim 3] Assay of the quinolone agent in saliva characterized by measuring the fluorescence which irradiates excitation light and produces saliva as a result under acid conditions.
[Claim 4] Assay of the quinolone agent characterized by measuring the fluorescence which irradiates excitation light and produces as a result the saliva containing the quinolone agent which has a pyrid benzoxazine frame under acid conditions.
[Claim 5] The quinolone agent which has a pyrid benzoxazine frame is 9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-. - Assay of 2 and the quinolone agent according to claim 4 which is a 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid.
[Claim 6] The quinolone agent which has a pyrid benzoxazine frame is S-(-) oxo-. - Assay of 2 and the quinolone agent according to claim 4 which is a 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid.
[Claim 7] Assay of the quinolone agent according to claim 4 whose quinolone agents which have a pyrid benzoxazine frame are the 10-(8-(S)-amino-6-azaspiro [3, 4] octane-6-IRU)-9-fluoro -2 and a 3-dihydro-3-(S)-methyl-7-oxo--7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the assay of a quinolone agent.
[0002]
[Background of the Invention] A derivative which has a fluorine in the 6th place (or the considerable grade) of a chemical structure top quinolone nucleus, and has a piperazine ring, a pyrrolidine ring, etc. in the 7th place, and the so-called quinolone agent are widely used as a broad spectrum antibiotic. Before long 9-fluoro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-- 2 and a 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (JP,57-46986,A) (it omits Following OFLX), i.e., oral quinolone agent ofloxacin In the Homo sapiens body, a metabolic turnover is hardly received but it accepts from mainly being excreted in urine with the non-changed body as a drug which is very easy to carry out an administration design. Recently, the opportunity prescribed by elderly people, especially the renal failure patient is also increasing. However, OFLX is a renal excretion mold and it has turned out that correction of an administration design and the monitoring of the concentration in blood are required about the patient to whom the kidney function is falling.
[0003]
[Problem(s) to be Solved by the Invention] The monitoring of a quinolone agent does not need a high-class device, but needs to obtain a result on a bed side for a short time.
[0004]
[Means for Solving the Problem] What is necessary is just to measure the fluorescence, since many of quinolone agents emit the fluorescence near 400-500nm. Furthermore, when excitation light is irradiated at a sample and fluorescence wavelength moves to a long wavelength side on some conditions, lightness becomes high to a naked eye and I think that the detection sensitivity in which fluorescence observation is possible is obtained with the naked eye. [0005] which measurement of the concentration in saliva can be enough performed among blood and urine if this is applied, and considers that the measurement is also possible by performing the comparison with the correlation sample in a naked eye instead of a fluorophotometer In the structure, the 3rd place is asymmetrical carbon, and OFLX is obtained by the usual process as racemic modification ((**) object specific rotation [alpha] D 0 degree). While this optically-active-substance 3S body ((-) object) has twice [about] as many antimicrobial activity as the (**) object, it is known compared with the (+) object that toxicity is low (JP,62-252790,A). This optically active substance, S-(-)-9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-- 2 and a 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (it abbreviates to DR-3355) can do a quantum similarly. Furthermore, one of the quinolone agents which have a pyrrolidine ring in the 7th place of a quinolone nucleus 10- () [8-] (S) The - friend no 6-azaspiro [3, 4] octane-6-IRU-9-fluoro -2, a 3-dihydro-3-(S)-methyl-7-oxo--7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (it abbreviates to DV-7751) The quantum of (JP,3-95176,A) can be carried out similarly.
[0006] that is, acidity -- it is -- further -- desirable -- pH 3-4 -- it is -- the quinolone agent (ciprofloxacin --) of others [wavelength / of OFLX / fluorescence] That it is in a merit side has

contributed the lightness in which fluorescence observation is possible to making it high with the naked eye. norfloxacin etc. -- comparing -- a long wave -- lone of the oxygen atom combined with the 8th place of a quinolone frame Since a pair electron is considered to participate in the resonating structure of a ring, a quantum is possible also for DR-3355 which have this structure, and DV-7751.

[0007] One of the oral quinolone agents and the concentration in saliva of OFLX correlate in serum concentration well (it can become the blood-drug-concentration monitoring method from Norikata Ichihara et al., Chemotherapy (Tokyo), 32,118-149, and a thing (1984) enough by detecting the OFLX concentration in saliva.).

[0008] The artificer completed the assay of the quinolone agent which measures the fluorescence which makes analyte containing a quinolone agent the source of an exposure for a fluorescent lamp etc. under acid conditions, irradiates excitation light, and is produced as a result here, as a result of repeating research. At this time, when the maximum fluorescence wavelength of the fluorescence of a quinolone agent was near 490nm to 550nm at the time of acidity, it found out that a quantum could also perform macro-scopic observation. The assay of the quinolone agent in saliva which measures the fluorescence which irradiates excitation light and furthermore produces saliva as a result under acid conditions was completed.

[0009]

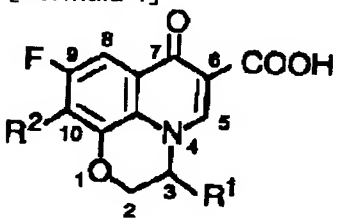
[Elements of the Invention] The assay of the quinolone agent characterized by this invention measuring the fluorescence which irradiates excitation light and produces the analyte containing a quinolone agent as a result under acid conditions is offered. If the maximum fluorescence wavelength is near 490nm to 550nm at the time of acidity in the case of this measurement, it is the assay in which macro-scopic observation also has a possible quantum.

[0010] As analyte containing a quinolone agent, blood, urine, saliva, etc. are mentioned, among these the assay of the quinolone agent in saliva characterized by measuring the fluorescence which irradiates excitation light under acid conditions and produces saliva as a result offers useful assay, when performing monitoring of a quinolone agent.

[0011] The assay of this invention is useful to a quinolone agent and the thing which especially has a pyrid benzoxazine frame. What has the structure of the following general formula as such quinolone can be mentioned (in addition, although it is made to represent by the thing of RS arrangement about the arrangement in the 3rd place, the thing of S arrangement (beta arrangement) is also contained.).

[0012]

[Formula 1]



[0013] Among a formula, R1 means a low-grade alkyl group, and especially its a methyl group is desirable. It has already become clear that the S arrangement of the configuration of this part is more desirable in respect of pharmacological activity.

[0014] R2 means a saturation nitrogen-containing heterocycle substituent. The magnitude of a ring has four to seven desirable membered-rings, and five membered-rings and its six membered-rings are especially desirable. Moreover, an oxygen atom, a sulfur atom, or two or more nitrogen atoms may also be included as a configuration atom of a ring like oxazolidine, a morpholine, thiazolidine, a thio morpholine, imidazolidine, pyrazolidine, and a piperazine. Especially as a saturation nitrogen-containing heterocycle substituent, a pyrrolidinyl radical and a piperazinyl radical may be desirable, and these may have the substituent further. Under the present circumstances, it is desirable that it is a substituent single in stereoisomerism.

[0015] As such a substituent, it is also a certain amino alkyl group and **5-permutation-2-oxo-

to also have ** substituent and to have a certain amino group and ** substituent. - They are polar groups, such as 1, a 3-JIOKI SOL-4-ylmethyl radical, or ** hydroxyl group. The shape of a straight chain, the shape of branching, and the annular alkyl group of the ** carbon numbers 1-6 can be mentioned again. Moreover, in the case of a polar group, you may combine with a saturation nitrogen-containing heterocycle substituent through the alkylene group of carbon numbers 1-6. Here, as a substituent of the amino group, an alkyl group, an acyl group, an acyloxy carbonyl group, etc. can be mentioned.

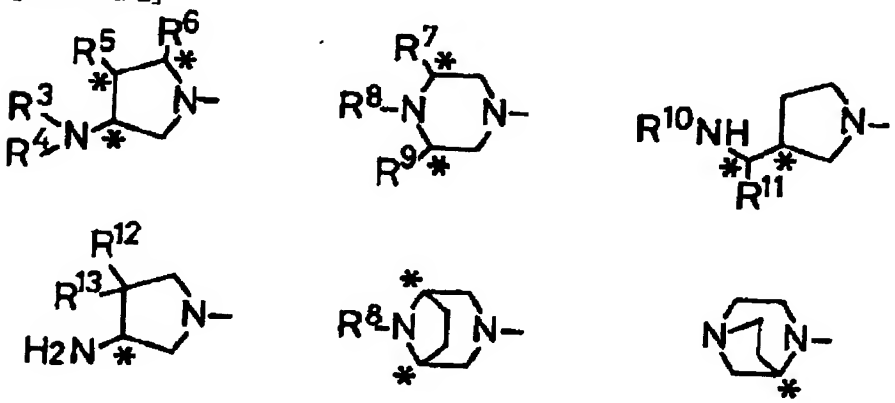
[0016] Especially as an above-mentioned polar group, non-permuted the amino group, an aminomethyl radical, 1-aminoethyl radical, and a hydroxyl group are desirable.

[0017] moreover -- as the alkyl group on a saturation nitrogen-containing heterocycle substituent -- methyl group an ethyl group, a propyl group, and an isopropyl group -- moreover -- gem- a dimethyl radical -- and -- As for a gem-diethyl radical etc., it is also still more desirable to become the saturation nitrogen-containing heterocycle substituent which these formed the cyclopropane ring and the cyclobutane ring and became a spiro ring system. Moreover, the bridge is constructed over the saturation nitrogen-containing heterocycle substituent of 4 to 7 membered-rings, and you may become a bicyclo annular saturation nitrogen-containing heterocycle substituent.

[0018] although it has the thing permuted especially by the amino group among these saturation nitrogen-containing heterocycle substituents, or the 2nd nitrogen atom -- an example -- ** -- if it carries out, the thing of the following structure can be shown.

[0019]

[Formula 2]



(Among a formula, R3, R4, R5, R6, R7, R8, R9, R10, R11, R12, and R13 mean independently a hydrogen atom or the alkyl group of carbon numbers 1-6, and R12 and R13 may join together, and they may form a polymethylene chain, and may form six membered-rings from three membered-rings.)

[0020] As an example of these substituents, 3-amino pyrrolidinyl radical, 3-methylamino pyrrolidinyl radical, 3-dimethylamino pyrrolidinyl radical, 3-ethylamino pyrrolidinyl radical, 3-propylamino pyrrolidinyl radical, 3-isopropylamino pyrrolidinyl radical, A 3-amino-4-methyl pyrrolidinyl radical, a 4-amino-2-methyl pyrrolidinyl radical, The 4-amino -2, 3-dimethyl pyrrolidinyl radical, a 3-methylamino-4-methyl pyrrolidinyl radical, A 4-methylamino-2-methyl pyrrolidinyl radical, 4-methylamino -2, 3-dimethyl pyrrolidinyl radical, A 3-dimethylamino-4-methyl pyrrolidinyl radical, a 4-dimethylamino-2-methyl pyrrolidinyl radical, 4-dimethylamino -2, 3-dimethyl pyrrolidinyl radical, 3-methyl piperazinyl radical, 4-methyl piperazinyl radical, 3, 4-dimethyl piperazinyl radical, 3, 5-dimethyl piperazinyl radical, 3, 4, a 5-trimethyl piperazinyl radical, 4-ethyl -3, 5-dimethyl piperazinyl radical, The 4-isopropyl -3, 5-dimethyl piperazinyl radical, 3-aminomethyl pyrrolidinyl radical, 3-methylamino methyl pyrrolidinyl radical, 3-(1-amino) ethyl pyrrolidinyl radical, 3-(1-methylamino) ethyl pyrrolidinyl radical, 3-(1-ethylamino) ethyl pyrrolidinyl radical, 3-(1-amino) propyl pyrrolidinyl radical, 3-(1-methylamino) propyl pyrrolidinyl radical, 3-amino pyrrolidinyl radical, the 4-amino -3, 3-dimethyl pyrrolidinyl radical, 7 - An amino-5-azaspiro [2, 4] heptane-5-IRU radical, 8-amino-6-azaspiro [3, 4] Octane-6-IRU radical,

A 1, 4-diazabicyclo [3, 2, 1] octane-4-IRU radical, 3, and 8-diazabicyclo [3, 2, 1] octane-3-IRU radical, The 8-methyl -3, a 8-diazabicyclo [3, 2, 1] octane-3-IRU radical, 8-ethyl -3, a 8-diazabicyclo [3, 2, 1] octane-3-IRU radical, etc. can be mentioned.
[0021] As a saturation nitrogen-containing heterocycle substituent which has substituents other than the amino group, moreover, for example 3-hide ROKISHI pyrrolidinyl radical, 3-mercapto pyrrolidinyl radical, A 3-hide ROKISHI-4-methyl pyrrolidinyl radical, a 3-mercapto-4-methyl pyrrolidinyl radical, Morpholino radical, thio morpholino radical, 2-methyl morpholino radical, 2-methylthio morpholino radical, 2, 6-dimethyl morpholino radical, 2, 6-dimethyl thio morpholino radical, 2, and 2-dimethyl morpholino radical, 2, and 2-dimethyl thio morpholino radical etc. can be mentioned.
[0022] This invention the saliva containing the quinolone agent which has a pyrid benzoxazine frame under acid conditions As a quinolone agent which offers the assay of the quinolone agent characterized by measuring the fluorescence which irradiates excitation light and is produced as a result, and has a pyrid benzoxazine frame further 9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-- 2 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid, S-(-) oxo-- 2 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid, And the assay of the 10-(8-(S)-amino-6-azaspiro [3, 4] octane-6-IRU)-9-fluoro -2 and the quinolone agent of a 3-dihydro-3-(S)-methyl-7-oxo--7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid It provides.
[0023] An example explains in detail below.
[0024]
[Example 1] What tore the extracting method A method-absorbent cotton of saliva, and was lightly rounded off to the globular form with a diameter of about 5mm was included in two to three-piece opening, was bit lightly, and saliva was included enough. It inquired, also when a candy, a citric acid, etc. were included in opening together with absorbent cotton at this time. Absorbent cotton was taken out with the pincettes, as beforehand shown in drawing 1 , it carried on the gauze of the transparence sample tube (the product made from polystyrene, the product made from assistance, No.17.452.722) which set gauze, and it covered as [stop / on both sides of gauze], and at-long-intervals alignment separation was carried out with the short form centrifuge (made in [Nihon Millipore] Cheevy Tan) for about 1 minute.
[0025] Direct saliva was extracted in the test tube from the healthy adult man B law at the purposes, such as recovery measurement of -OFLX.
[0026] Although it is B law, for those who are extracted, the easiest approach as a method of extracting saliva is pain, and in order to extract the saliva of an initial complement for a short time, it needs skill to some extent. 1 Hara and others includes the paper disc of bioassay in opening, and is extracting saliva (Norikata Ichihara et al., Chemotherapy (Tokyo), 32,118-149, (1984)). Although this approach is comparatively easy, it cannot extract a complement to fluorescence detection, and recovery from a filter paper is difficult for it. It is 0.3ml easily, without almost giving pain to those who are extracted in A law. Extent was able to be obtained in a short time. In case saliva was extracted, by including a candy, a citric acid, etc. in opening together, secretion of saliva was promoted and was still easier to extract. Unlike the saliva extracted in the direct test tube for a while, the description of the saliva extracted by this approach had little protein with high viscosity comparatively peculiar to saliva.
[0027]
[Example 2] It is the solution (solvent 1:0.1M phosphate buffer solution (pH7.0).) of various pH about the examination OFLX of fluorescence observation conditions. 2:0. 05M acetic-acid buffer solution (pH4.0). 3:0. 04M Phosphate buffer solution (pH3). 4:5M It dissolved in the sulfuric acid by 10microg [/ml] concentration, and the fluorescence spectrum was measured with the Hitachi 650-60 mold spectrophotofluorometer (Hitachi). On the occasion of measurement, the solution of pH7 performed the press can, the sensibility of a spectrophotofluorometer was standardized, and the sample was measured by this sensibility after that. A result is shown in drawing 2 .
[0028] As for the compound of fluorescence, it is known that the wavelength of fluorescence and reinforcement generally change with differences in the dissociation condition. Although the excitation spectrum seldom changed when pH changed to 4 from 7 about OFLX, no less than

40nm of fluorescence spectra was shifted to long wavelength. The fluorescence spectrum seldom changed as for an acidity side more than this after this, and fluorescence intensity became max by pH3, and it decreased by the acidity side rather than it.
[0029] with the naked eye, change of such a fluorescence spectrum is observed as change of the color tone of fluorescence -- having -- ** in pH7 -- since it was blue, it changed to the yellowish green in an acid field. The lightness sensed with this change with the naked eye increased remarkably by the acidity side. therefore, ** by reflection of the exposure light of a fluorescent lamp when a saliva sample is set to pH3 and it irradiates with a fluorescent lamp (UVGL- 25 molds, UVP, Inc. CA, USA) -- since it was blue, when changing to clearly distinguishable yellowish green and observing with the naked eye, the sensibility of quinolone agent detection was able to be raised and dependability was able to be raised.
[0030]
[Example 3] B of the creation example 2 of a standard addition sample -- for the transparence sample tube, it was easy to add 10microl of the water solution (concentration : 4, 10, and 20 or 40,100microg/(ml)) of OFLX, 0.2ml mixed at a time with the saliva extracted by law, and it considered as each [the concentration 0.2 of OFLX, 0.5, 1.0, 2.0, and 5.0microg //ml] sample. It is 0.04M to this. 0.2ml (pH3) of phosphate buffer solutions was added, it mixed with them, at-long-intervals alignment separation was carried out with the short form centrifuge for about 10 minutes, and protein was settled. Black Kent flock paper was made into the background, the sample tube was irradiated with the fluorescent lamp (UVGL- 25 molds, UVP, Inc. CA, USA), and fluorescence was observed with the naked eye. A result is shown in drawing 3 . From drawing 3 , the concentration of OFLX could be detected quantitatively with the naked eye, and has been detected also by the 0.2microg [/ml] concentration in saliva. Although it was thought that the limit of detection in a naked eye had individual difference, it was 0.2micro aboutg/ml.
[0031] Concentration in saliva is made [OFLX] Homo sapiens in 0.4 to 0.7 microg/ml by 100mg time amount. When oral single-dose administration is carried out, it is after [administration] 2. (Norikata Ichihara et al., Chemotherapy (Tokyo), 32,118-149 (1984)) Moreover, when 200mg is pitched in successive games every 12 hours, the plasma concentration in front of the 2nd administration is reported for the concentration in front of 0.53microg [ml] /and the 5th administration to be 1.19microg/ml (Couraud, L. et al., Drugs, 34, (Suppl.1), 37-38, (1987)). Since it is expected that the concentration in saliva at this time is comparable as plasma concentration, a monitor is possible at the above-mentioned detection sensitivity. When a dose is increased, becoming the concentration in saliva higher than this is expected, and the detection sensitivity in a naked eye is sufficient sensibility, in order to prevent are recording of OFLX by clinical and to use it.
[0032] B of an example 2 -- 1ml of saliva extracted by law -- an OFLX solution -- adding -- 0.5-0.004microg/ml -- carrying out -- further -- 4ml (pH3) of 0.04M phosphate buffer solutions was added, the sample was adjusted, and it put into the methacrylate cuvette (Aldrich company catalog No.Z18801-8) of UV-clade, and measured with the Jasco FP777 mold spectrophotofluorometer. The result when using 500nm for excitation wavelength at 303nm, 330nm and 360nm, and fluorescence wavelength was as shown in Table 1.
[0033]
[Table 1]

唾液中OFLX濃度	蛍光強度（出力表示）		
（μg/ml）	303 nm	330 nm	360 nm
0.000	13.84	6.576	5.823
0.004	67.66	31.94	14.74
0.02	309.3	148.5	58.66
0.1	1471	714.1	273.5
0.5	6921	3445	1328
（r：相関係数）	r=0.999939	r=0.999981	r=0.999995

[0034]
[Example 4] OFLX was dissolved in the saliva extracted by B law of the measurement example 1 of recovery at 5microg [/ml] concentration. Absorbent cotton was absorbed with this solution, centrifugal was put in and carried out to the container liner of a transparence sample tube, and filtrate was obtained. The OFLX concentration in this filtrate and the original saliva was measured by the HPLC method (Matsubayashi K. et al., Journal of Chromatography, 495,354-357 (1989)), and the recovery of actuation by absorption with absorbent cotton and recovery was searched for.
[0035] In addition, it is in measurement of an excitation spectrum. It detected on the maximum fluorescence wavelength and carried out to measurement of a fluorescence spectrum by exciting on the maximum excitation wavelength.
[0036] The recovery of OFLX was 93.5**5 and 4% at the appearance shown in Table 1. That is, absorbent cotton was made to absorb saliva and OFLX(s) were quantitatively collected by the approach (A of an example 1 law) of collecting according to centrifugal separation.
[0037]
[Table 2]
回収率の測定

試行回数	1	2	3	平均
回収%	102.8	95.6	110.7	93.4 ±5.4

[0038]
[Example 5] What is necessary is just to perform quantum DR-3355 of DR-3355 as follows for carrying out a quantum.
[0039] Saliva is separated according to A law of an example 1. It is 0.1ml in 0.2ml of saliva. 0.04M phosphoric-acid buffer 1 liquid (pH3) is added, and it considers as acid conditions. On the other hand, a standard addition sample is created according to an example 3. A sample tube is irradiated with a fluorescent lamp by making black Kent flock paper into a background, and fluorescence is observed with the naked eye. The saliva concentration of DR-3355 is decided as compared with the fluorescence of a standard addition sample.
[0040] Moreover, it is also possible to extract saliva by the capillary tube, a syringe, etc. besides example 1. If it dozens of micro last l Is, it is observable by carrying as waterdrop on Saran Wrap etc. with the naked eye. Therefore, a quantum will be made if there divisor [of 10micro] l Is also saliva. A quantum is possible by observing the fluorescence in saliva of a quinolone agent and comparing with a correlation sample by putting a sample into the thin slit between the waterdrop formation on Saran Wrap, or two glass plates, if the observation under acid conditions is also little, covering the bottom of Saran Wrap or a glass plate with black flock paper, and irradiating

with a fluorescent lamp.
[0041]
[Example 6] Like examination -1 example 2 of the fluorescence observation conditions of other oral quinolone agents The 10-(4-amino -3, 3-dimethyl-1-pyrrolidinyl)-9-fluoro -2, 3-dihydro-3-(S)-3-methyl-7-oxo-- a 7H-pyrid [1, 2, 3-de] [1, 4]-benzoxazine-6-carboxylic acid (it abbreviates to DV-7777) The solution of two sorts of pH, 1. 0.1M phosphate buffer solution (pH7.0) or 2. 0.04M It dissolved in the phosphate buffer solution (pH3) at 2microg [/ml] concentration, and the fluorescence spectrum was measured with the Hitachi 650 mold spectrophotofluorometer. The press can was performed at every measurement and the sensibility of a spectrophotofluorometer was standardized. A result is shown in drawing 4 .
[0042] Although the excitation spectrum seldom changed when pH changed to 3 from 7 like OFLX as for DV-7777, no less than 40nm of fluorescence spectra was shifted to long wavelength.
[0043] with the naked eye, change of such a fluorescence spectrum is observed as change of the color tone of fluorescence -- having -- ** in pH7 -- since it was blue, it changed to yellowish green in the acid field. The lightness sensed with this change with the naked eye increased remarkably by the acidity side.
[0044] Furthermore, when it was made the bottom of an acid condition, it irradiated with the fluorescent lamp and the limit of detection by the naked eye was measured, it was 0.1microg/ml. It was equivalent in whether this is a little more highly sensitive than the case of OFLX.
[0045]
[Example 7] Like examination -2 example 2 of the fluorescence observation conditions of other oral quinolone agents The 10-(8-(S)-amino-6-azaspiro [3, 4] octane-6-IRU)-9-fluoro -2 and a 3-dihydro-3-(S)-methyl-7-oxo--7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (DV-7751) the solution of two sorts of pH, and a 0.1M phosphate buffer solution (pH7.0) -- or -- It dissolved in the 0.04M phosphate buffer solution (pH3) at 1microg [/ml] concentration, and the fluorescence spectrum was measured with the Hitachi 650 mold spectrophotofluorometer. The press can was performed at every measurement and the sensibility of a spectrophotofluorometer was standardized. A result is shown in drawing 5 .
[0046] Although the excitation spectrum seldom changed when pH changed to 3 from 7 like DV-7777 as for DV-7751, no less than 45nm of fluorescence spectra was shifted to long wavelength. Change of this fluorescence spectrum was the same as that of DV-7777. When it was made the bottom of an acid condition, it irradiated with the fluorescent lamp and the limit of detection by the naked eye was measured, it was 0.2microg/ml.
[0047]
[Effect of the Invention] In a short time and about 5 minutes, this assay can know a result and can respond to need promptly in a bedside. Absorbent cotton is included in a patient at opening, it takes out, the buffer solution is added, it considers as acidity, and actuation also only measures fluorescence intensity. When measuring by performing macro-scopic observation, in a facility, just a fluorescent lamp is enough. Therefore, it measures quickly at a clinical bedside and can respond to the purpose to adjust in consideration of a dose.
[0048]

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EFFECT OF THE INVENTION

[Effect of the Invention] In a short time and about 5 minutes, this assay can know a result and can respond to need promptly in a bedside. Absorbent cotton is included in a patient at opening, it takes out, the buffer solution is added, it considers as acidity, and actuation also only measures fluorescence intensity. When measuring by performing macro-scopic observation, in a facility, just a fluorescent lamp is enough. Therefore, it measures quickly at a clinical bedside and can respond to the purpose to adjust in consideration of a dose.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] The monitoring of a quinolone agent does not need a high-class device, but needs to obtain a result on a bed side for a short time.

[Translation done.]

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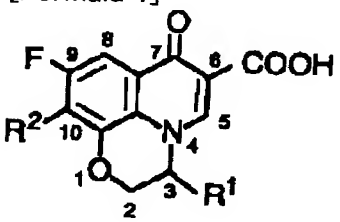
MEANS

[Means for Solving the Problem] What is necessary is just to measure the fluorescence, since many of quinolone agents emit the fluorescence near 400-500nm. Furthermore, when excitation light is irradiated at a sample and fluorescence wavelength moves to a long wavelength side on some conditions, lightness becomes high to a naked eye and I think that the detection sensitivity in which fluorescence observation is possible is obtained with the naked eye. [0005] which measurement of the concentration in saliva can be enough performed among blood and urine if this is applied, and considers that the measurement is also possible by performing the comparison with the correlation sample in a naked eye instead of a fluorophotometer In the structure, the 3rd place is asymmetrical carbon, and OFLX is obtained by the usual process as racemic modification ((**) object specific rotation [alpha] D 0 degree). While this optically-active-substance 3S body ((-) object) has twice [about] as many antimicrobial activity as the (**) object, it is known compared with the (+) object that toxicity is low (JP,62-252790,A). This optically active substance, S-(-)-9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-- 2 and a 3-dihydro-7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (it abbreviates to DR-3355) can do a quantum similarly. Furthermore, one of the quinolone agents which have a pyrrolidine ring in the 7th place of a quinolone nucleus 10- () [8-] (S) The - friend no 6-azaspiro [3, 4] octane-6-IRU-9-fluoro -2, a 3-dihydro-3-(S)-methyl-7-oxo--7H-pyrid [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (it abbreviates to DV-7751) The quantum of (JP,3-95176,A) can be carried out similarly.
[0006] that is, acidity -- it is -- further -- desirable -- pH 3-4 -- it is -- the quinolone agent (ciprofloxacin --) of others [wavelength / of OFLX / fluorescence] That it is in a merit side has contributed the lightness in which fluorescence observation is possible to making it high with the naked eye. norfloxacin etc. -- comparing -- a long wave -- lone of the oxygen atom combined with the 8th place of a quinolone frame Since a pair electron is considered to participate in the resonating structure of a ring, a quantum is possible also for DR-3355 which have this structure, and DV-7751.
[0007] One of the oral quinolone agents and the concentration in saliva of OFLX correlate in serum concentration well (it can become the blood-drug-concentration monitoring method from Norikata Ichihara et al., Chemotherapy (Tokyo), 32,118-149, and a thing (1984) enough by detecting the OFLX concentration in saliva.).
[0008] The artificer completed the assay of the quinolone agent which measures the fluorescence which makes analyte containing a quinolone agent the source of an exposure for a fluorescent lamp etc. under acid conditions, irradiates excitation light, and is produced as a result here, as a result of repeating research. At this time, when the maximum fluorescence wavelength of the fluorescence of a quinolone agent was near 490nm to 550nm at the time of acidity, it found out that a quantum could also perform macro-scopic observation. The assay of the quinolone agent in saliva which measures the fluorescence which irradiates excitation light and furthermore produces saliva as a result under acid conditions was completed.
[0009]
[Elements of the Invention] The assay of the quinolone agent characterized by this invention measuring the fluorescence which irradiates excitation light and produces the analyte containing

a quinolone agent as a result under acid conditions is offered. If the maximum fluorescence wavelength is near 490nm to 550nm at the time of acidity in the case of this measurement, it is the assay in which macro-scopic observation also has a possible quantum.
[0010] As analyte containing a quinolone agent, blood, urine, saliva, etc. are mentioned, among these the assay of the quinolone agent in saliva characterized by measuring the fluorescence which irradiates excitation light under acid conditions and produces saliva as a result offers useful assay, when performing monitoring of a quinolone agent.
[0011] The assay of this invention is useful to a quinolone agent and the thing which especially has a pyrid benzoxazine frame. What has the structure of the following general formula as such quinolone can be mentioned (in addition, although it is made to represent by the thing of RS arrangement about the arrangement in the 3rd place, the thing of S arrangement (beta arrangement) is also contained.).
[0012]
[Formula 1]



[0013] Among a formula, R1 means a low-grade alkyl group, and especially its a methyl group is desirable. It has already become clear that the S arrangement of the configuration of this part is more desirable in respect of pharmacological activity.
[0014] R2 means a saturation nitrogen-containing heterocycle substituent. The magnitude of a ring has four to seven desirable membered-rings, and five membered-rings and its six membered-rings are especially desirable. Moreover, an oxygen atom, a sulfur atom, or two or more nitrogen atoms may also be included as a configuration atom of a ring like oxazolidine, a morpholine, thiazolidine, a thio morpholine, imidazolidine, pyrazolidine, and a piperazine. Especially as a saturation nitrogen-containing heterocycle substituent, a pyrrolidinyl radical and a piperazinyl radical may be desirable, and these may have the substituent further. Under the present circumstances, it is desirable that it is a substituent single in stereoisomerism.
[0015] As such a substituent, it is also a certain amino alkyl group and **5-permutation-2-oxo- to also have ** substituent and to have a certain amino group and ** substituent. - They are polar groups, such as 1, a 3-JIOKI SOL-4-ylmethyl radical, or ** hydroxyl group. The shape of a straight chain, the shape of branching, and the annular alkyl group of the ** carbon numbers 1-6 can be mentioned again. Moreover, in the case of a polar group, you may combine with a saturation nitrogen-containing heterocycle substituent through the alkylene group of carbon numbers 1-6. Here, as a substituent of the amino group, an alkyl group, an acyl group, an acyloxy carbonyl group, etc. can be mentioned.
[0016] Especially as an above-mentioned polar group, non-permuted the amino group, an aminomethyl radical, 1-aminoethyl radical, and a hydroxyl group are desirable.
[0017] moreover -- as the alkyl group on a saturation nitrogen-containing heterocycle substituent -- methyl group an ethyl group, a propyl group, and an isopropyl group -- moreover -- gem- a dimethyl radical -- and -- As for a gem-diethyl radical etc., it is also still more desirable to become the saturation nitrogen-containing heterocycle substituent which these formed the cyclopropane ring and the cyclobutane ring and became a spiro ring system. Moreover, the bridge is constructed over the saturation nitrogen-containing heterocycle substituent of 4 to 7 membered-rings, and you may become a bicyclo annular saturation nitrogen-containing heterocycle substituent.
[0018]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Fig. 1 is an explanatory view of the separation method of a saliva sample.
[Drawing 2] Fig. 2 is drawing of the excitation spectrum and fluorescence spectrum of OFLX.
[Drawing 3] Fig. 3 is drawing showing the fluorescence intensity of the OFLX correlation sample in saliva.
[Drawing 4] Fig. 4 is drawing of the excitation spectrum and fluorescence spectrum of DV-7777.
[Drawing 5] Fig. 5 is drawing of the excitation spectrum and fluorescence spectrum of DV-7751.

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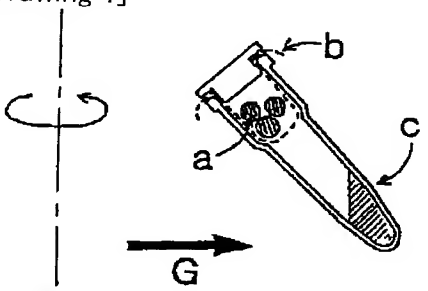
DRAWINGS

[Drawing 1]



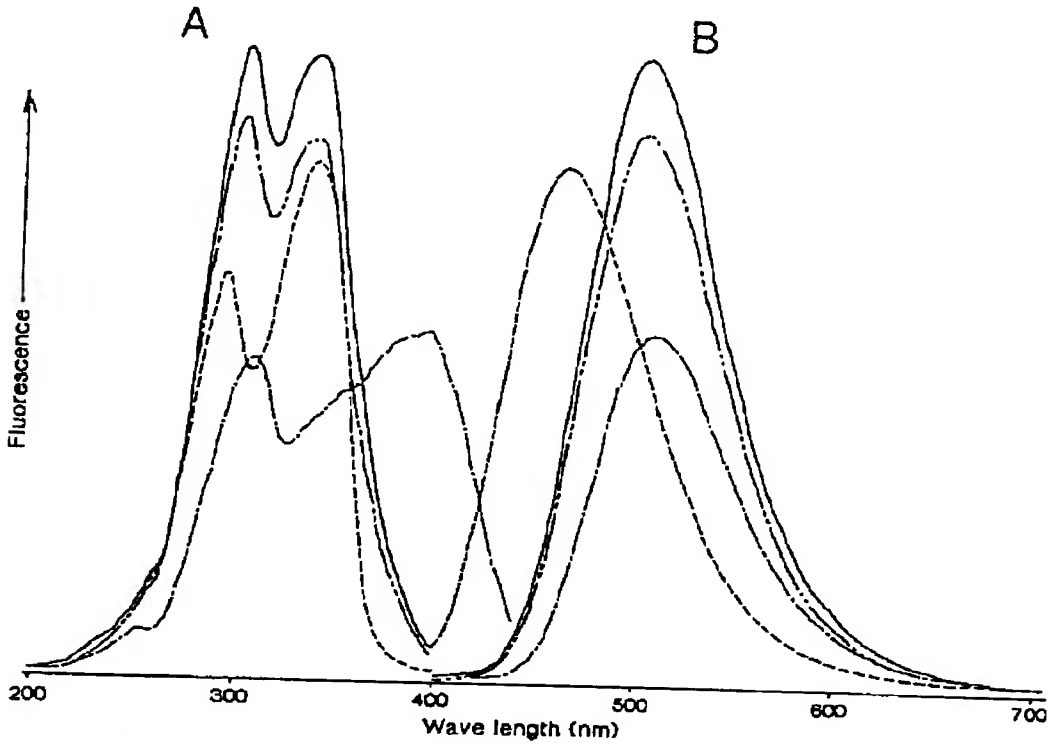
a：脱脂綿 b：ガーゼ c：サンプルチューブ G：遠心力

[Drawing 3]

a：0, b：0.2, c：0.5, d：1.0, e：2.0, f：5.0
(μg/ml)

サンプルチューブ内の印影が黒く濃くなるほど蛍光強度が強いことを示す。

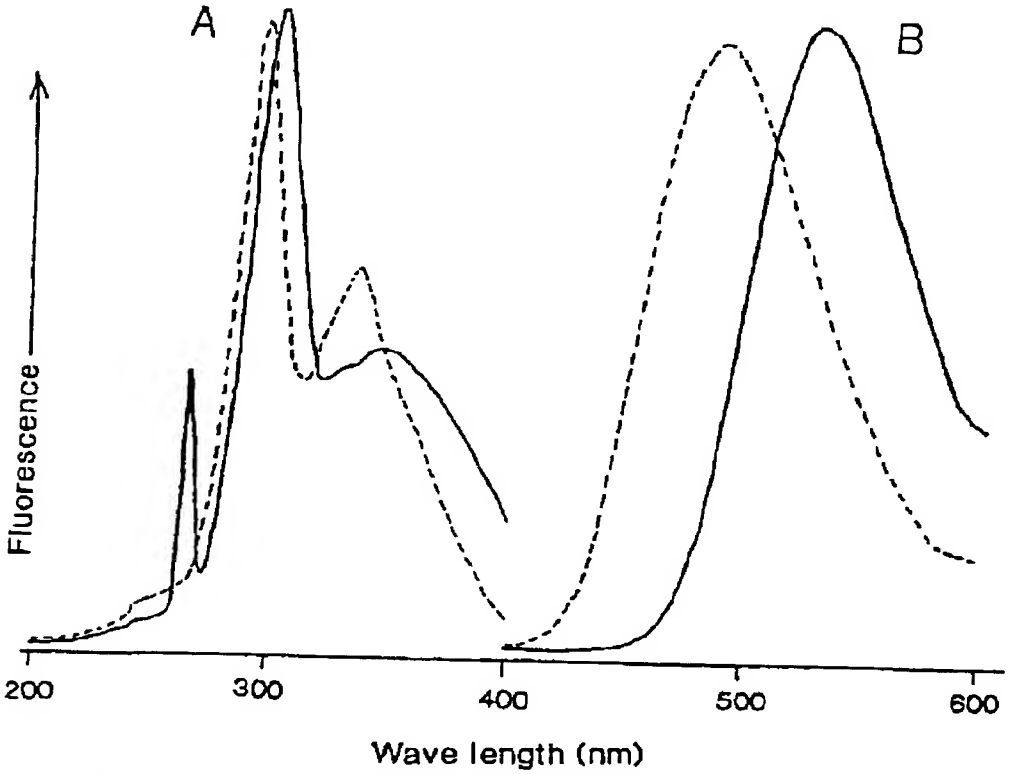
[Drawing 2]



A：励起スペクトル　　B：蛍光スペクトル

OFLXを5M硫酸中で測定（----）、0.04Mリン酸緩衝液pH3中で測定（——）、0.05M酢酸緩衝液pH4中で測定（-·--）、および0.1Mリン酸緩衝液pH7中で測定（-----）。

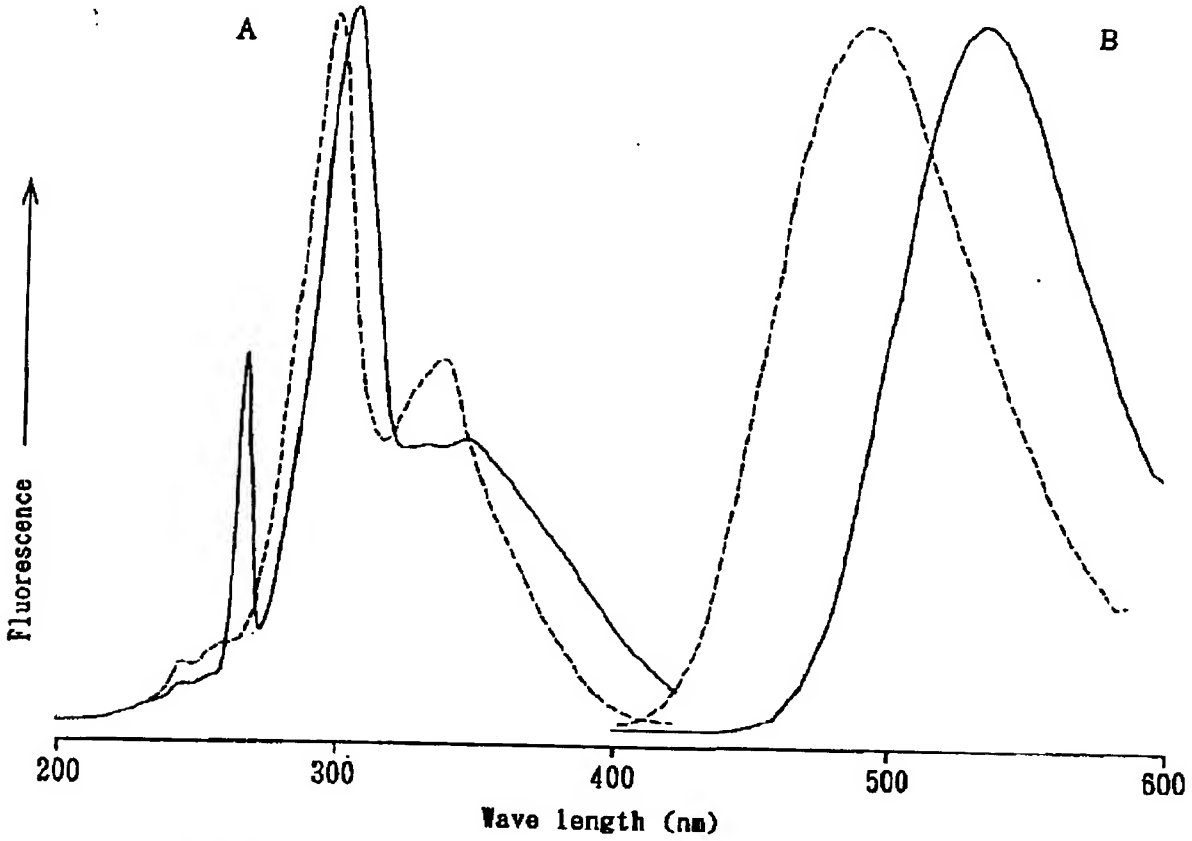
[Drawing 4]



A：励起スペクトル　　B：蛍光スペクトル

0.04Mリン酸緩衝液pH3中で測定（——）および0.1Mリン酸緩衝液pH7中で測定（- - - -）。

[Drawing 5]



A: 励起スペクトル　　B: 蛍光スペクトル．
0.04Mリン酸緩衝液pH3中で測定(———)および0.1Mリン酸緩衝液pH7中で測定(------)。

[Translation done.]